UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF
PREVENTION, PESTICIDES, AND
TOXIC SUBSTANCES

PC Code: 024875
DP Barcode: 354077

MEMORANDUM

June 25, 2008

SUBJECT: **Spiromesifen:** Transmittal of Data Evaluation Record for a Fate Study

TO: Amer Al-Mudallal, Risk Manager Reviewer
John Hebert, Risk Manager
IRB/RD (7505P)

FROM: Greg Orrick, Environmental Scientist Greg Orrick 6-25-08
ERB4/EFED (7507P)

THRU: Betsy Behl, Chief DBell 6-26-08
ERB4/EFED (7507P)

This memo is to inform you that one (1) DER for spiromesifen is finalized. Study type and MRID is provided in Table 1 below.

Table 1. Spiromesifen Study Type, MRID, and Electronic File Name.

Study Type	MRID	Electronic File Name	Classification
Non-guideline/ Aerobic Aquatic Metabolism	47133102	024875 47133102 Non-guideline.doc 024875 47133102 Non-guideline Calculations.xls 024875 47133102 Non-guideline Figures.pdf	Supplemental

Data Evaluation Record on the aerobic biotransformation of spiromesifen in water-sediment system

PMRA Submission Number {.....} EPA MRID Number 47133102

Data Requirement:	PMRA Data Code:	
	EPA DP Barcode:	354077
	OECD Data Point:	
	EPA Guideline:	Non-guideline/835.4300

Test material:

Common name:	Spiromesifen.
Chemical name:	
IUPAC name:	3-Mesityl-2-oxo-1-oxaspiro[4.4]non-3-en-4-yl 3,3-dimethylbutyrate.
CAS name:	2-Oxo-3-(2,4,6-trimethylphenyl)-1-oxaspiro[4.4]non-3-en-4-yl 3,3-dimethylbutanoate.
CAS No:	283594-90-1.
Synonyms:	C-798D, BSN 2060.
SMILES string:	C32(CCCC3)C(OC(=O)CC(C)(C)C)=C(C1C(C)=CC(C)=CC=1C)C(=O)O2 (EPI Suite, v3.12 SMILES string from ISIS .MOL). O=C(CC(C)(C)C)OC1=C(c2c(cc(cc2C)C)C)C(=O)OC11CCCC1 (SMILES String provided by study author).

Primary Reviewer: Dana Worcester
Cambridge Environmental

Signature:
Date: 07/12/07

Secondary Reviewer: Kathleen Ferguson
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Final Reviewer: Greg Orrick
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Signature: Greg Orrick
Date: 06/25/08

Company Code:
Active Code:
Use Site Category:
EPA PC Code: 024875

CITATION: Shepherd, J.J and J.G. Allan. 2007. Fate of spiromesifen-dihydrofuranone-3-^{14}C applied to a laboratory sediment followed by flooding and incubated for 28 days. Unpublished study performed by Bayer CropScience, Stilwell, Kansas; sponsored and submitted by Bayer CropScience, Research Triangle Park, North Carolina. Bayer Study and Report No.: MEBSX019. Experiment started February 13, 2007, and terminated March 23, 2007 (p. 6). Final report issued May 1, 2007.

Data Evaluation Record on the aerobic biotransformation of spiromesifen in water-sediment system

PMRA Submission Number {.....} EPA MRID Number 47133102

Data Requirement:	PMRA Data Code:	
	EPA DP Barcode:	D340140
	OECD Data Point:	
	EPA Guideline:	162-4

Test material:

Common name:	Spiromesifen.
Chemical name:	
IUPAC name:	3-Mesityl-2-oxo-1-oxaspiro[4.4]non-3-en-4-yl 3,3-dimethylbutyrate.
CAS name:	2-Oxo-3-(2,4,6-trimethylphenyl)-1-oxaspiro[4.4]non-3-en-4-yl 3,3-dimethylbutanoate.
CAS No:	283594-90-1.
Synonyms:	C-798D, BSN 2060.
SMILES string:	C32(CCCC3)C(OC(=O)CC(C)(C)C)=C(C1C(C)=CC(C)=CC=1C)C(=O)O2 (EPI Suite, v3.12 SMILES string from ISIS .MOL). O=C(CC(C)(C)C)OC1=C(c2c(cc(cc2C)C)C)C(=O)OC11CCCC1 (SMILES String provided by study author).

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CITATION: Shepherd, J.J and J.G. Allan. 2007. Fate of spiromesifen-dihydrofuranone-3-^{14}C applied to a laboratory sediment followed by flooding and incubated for 28 days. Unpublished study performed by Bayer CropScience, Stilwell, Kansas; sponsored and submitted by Bayer CropScience, Research Triangle Park, North Carolina. Bayer Study and Report No.: MEBSX019. Experiment started February 13, 2007, and terminated March 23, 2007 (p. 6). Final report issued May 1, 2007.

EXECUTIVE SUMMARY

The biotransformation of [dihydrofuranone-3-^{14}C]-labeled 3-mesityl-2-oxo-1-oxaspiro[4.4]non-3-en-4-yl 3,3-dimethylbutyrate (spiromesifen; radiochemical purity 100%) was studied in a spring water-loamy sand sediment (water pH 6.9, dissolved organic carbon 48.1 mg/L; sediment pH 5.1-5.8, organic carbon 3.5%). The sediment was created by mixing peat moss, white quartz sand, kaolinite clay, and calcium carbonate, and was flooded with a mixture of deionized sterilized spring and municipal waters according to OECD Guideline 218 (Sediment-Water Chironomid Toxicity Test). The sediment samples were treated prior to flooding at 0.09 mg a.i./L, mixed for 30 minutes, flooded, and incubated for 28 days under aerobic conditions in darkness at 20 ± 1°C. The sediment:water ratio used was *ca.* 1:3.5 (100 g dry wt. sediment:349 mL water). This experiment was not intended to fulfill a guideline requirement, but to support the request for a waiver of the Sediment Toxicity Study (Draft Guideline 850.1790, Part 2). The experiment was conducted in compliance with USEPA GLP Standards 40 CFR, Part 160. The test system consisted of cylindrical glass flasks (500 mL, 7.2 cm i.d.) that were attached to a continuous flow-through volatile trapping system; humidified air was bubbled (10-20 mL/minute) through the water layer in the sample flasks, then through one tube of ethanolamine for the collection of volatiles. Single samples (entire flasks) were collected after 0, 1, 3, 7, 12, 17, 22 and 28 days of incubation. The water was decanted and filtered, and aliquots were analyzed using LSC and HPLC. The sediment was transferred to a Buchner funnel and filtered under vacuum to remove pore water; the pore water was analyzed using LSC. The sediment was extracted with acetonitrile:water with 0.1% formic acid (9:1, v:v) two or three times by shaking at ambient temperatures and twice using a microwave extraction at 80°C. Aliquots of the soil extracts were analyzed using LSC and HPLC. [^{14}C]Residues were identified by comparison to unlabeled reference standards of spiromesifen and 4-hydroxy-3-(2,4,6-trimethylphenyl)-1-oxaspiro[4.4]non-3-en-2-one (BSN2060-enol) that were cochromatographed with the samples. The trapping solution was analyzed using LSC, and the extracted soil was analyzed using LSC following combustion.

During the study, the temperature was maintained at 20 ± 0.3°C. The pH of the water increased from 6.7-6.9 to 7.8, and the pH of the sediment from 5.6-5.9 to 6.7. The dissolved oxygen content ranged from 3.8-6.1 mg/L; redox potentials were not reported. The viability of the test system was not determined.

Overall recoveries of [^{14}C]residues averaged 98.9 ± 1.8% (range 95.8-100.6%) of the applied with no pattern of decline over time. Total [^{14}C]residues moved from the sediment to the water with distribution ratios (sediment:water) of *ca.* 66:1 at time 0, 17:1 at 7 days, and 4:1 at 28 days. [^{14}C]Spiromesifen was almost entirely associated with the sediment throughout the study.

In the total system (water plus sediment), [^{14}C]spiromesifen decreased from 92.1-93.8% of the applied at 0 through 7 days posttreatment to 74.9% at 28 days (study termination). In the sediment, [^{14}C]spiromesifen decreased from 92.1-93.1% of the applied at 0-7 days posttreatment to 74.9% at 28 days. In the water layer, [^{14}C]spiromesifen was ≤0.7% at all sampling intervals.

Based on first order linear regression analysis (Excel 2000) and nonlinear analysis (SigmaPlot v 9), spiromesifen dissipated in the sediment and total system with half-lives of 80.6 days and 82.5 days, respectively. These half-lives are of uncertain value because they are extrapolated well beyond the duration of the study. A half-life was not calculated for the water layer. DT50 values were not observed in the water, sediment, or total system.

The only major transformation product was 4-hydroxy-3-(2,4,6-trimethylphenyl)-1-oxaspiro[4.4]non-3-en-2-one (BSN2060-enol), which increased to a maximum 22.0% of the applied in the total system at 28 days posttreatment (study termination); maximum concentrations were 16.6% in the water at 28 days and 6.2% in the sediment at 22 days. No minor transformation products were identified. Unidentified [^{14}C]residues were isolated only from the water, at a maximum of 3.5% of the applied. Extractable [^{14}C]residues increased from 92.5% at time 0 to 94.1% at 3 days and then decreased to 80.3% of the applied at 28 days. Nonextractable [^{14}C]residues decreased from 1.9% at time 0 to 0.3-0.5% at all other sampling intervals. No volatiles were detected in the ethanolamine trap.

Based on the results of the study, spiromesifen degrades to 4-hydroxy-3-(2,4,6-trimethylphenyl)-1-oxaspiro[4.4]non-3-en-2-one (BSN2060-enol) and several minor transformation products. BSN2060-enol moves into the water, while spiromesifen remaines primarily associated with the sediment.

Results Synopsis:

Test system used: Water-loamy sand sediment.

Linear half-life in water:	Not determined.
Linear half-life in sediment:	80.6 days (r^2 = 0.9300).
Linear half-life in the total system:	80.6 days (r^2 = 0.9249).
Non-linear half-life in water:	Not determined.
Non-linear half-life in sediment:	82.5 days (r^2 = 0.9995).
Non-linear half-life in total system:	82.5 days (r^2 = 0.9994).
Observed DT50 in water:	Not observed.
Observed DT50 in sediment:	Not observed.
Observed DT50 in total system:	Not observed.

Major transformation products:
4-hydroxy-3-(2,4,6-trimethylphenyl)-1-oxaspiro[4.4]non-3-en-2-one (BSN2060-enol).
Minor identified transformation products:
None.

Study Acceptability: This study is classified as **supplemental.** No significant deviations from good scientific practices were noted. This experiment was not intended to fulfill a guideline

requirement, but to support the request for a waiver of the Sediment Toxicity Study (Draft Guideline 850.1790, Part 2). The study was terminated at 28 days posttreatment, at which time 74.9% of the applied spiromesifen remained undegraded.

I. MATERIALS AND METHODS

GUIDELINE FOLLOWED: The study was intended to support the request for a waiver of the Sediment Toxicity Study (Draft Guideline 850.1790, Part 2) and was not designed to satisfy a Subdivision N guideline (p. 14). Significant deviations from the objectives of Subdivision N guidelines were:

The study was terminated at 28 days posttreatment, at which time 74.9% of the applied remained undegraded.

The water and loamy sand media were created in the lab rather than being naturally occurring.

COMPLIANCE: This study was conducted in compliance with USEPA GLP Standards (p. 14). Signed and dated Data Confidentiality, GLP, Quality Assurance and Certificate of Authenticity statements were provided (pp. 2-5).

A. MATERIALS:

1. Test Materials		[Dihydrofuranone-3-^{14}C]spiromesifen (p. 15).
Chemical Structure:		See DER Attachment 1.
Description:		Technical; physical state not reported.
Purity:	Radiochemical purity:	100% (p. 15).
	Batch No.	C798D.
	Analytical purity:	Not reported.
	Specific activity:	54.4 mCi/mMol.
	Location of the radiolabel:	On the 3 carbon of the dihydrofuranone chain.
Storage conditions of test chemicals:		Test substance was dissolved in acetonitrile and stored at -20°C (p. 15).

Physico-chemical properties of spiromesifen:

Parameter	Value	Comment
Molecular weight	370.49 g/mol.	
Molecular formula	$C_{23}H_{30}O_4$	
Water Solubility	0.13 mg/L	
Vapor Pressure	7×10^{-6} Pa 1×10^{-5} Pa	At 20°C. At 25°C.
UV Absorption	Not reported.	
pK_a	Not reported.	
Henry's Law	2×10^{-2} Pa x m^3/mol	
log K_{ow}	4.55	
Stability of compound at room temperature	Not reported.	

Data obtained from p. 15 of the study report.

2. Water-sediment collection, storage and properties

Table 1: Description of water-sediment collection and storage

Description		Details
Geographic location.		The sediment was artificially created and the water was a mixture of municipal water and spring water (spring location not reported)
Pesticide use history at the collection sites		Not applicable.
Collection date (creation date)		Not reported.
Collection procedures for:	Water:	Spring water was collected from a spring box, filtered (5 µ bag and activated carbon filters, and 1 µ cartridge) and UV sterilized. The spring water was mixed with purified (reverse osmosis) municipal water.
	Sediment:	Not applicable.
Sampling depth for:	Water:	Not applicable.
	Sediment:	Not applicable.
Storage conditions		Not reported.
Storage length		Not reported.
Preparation	Water:	Filtered (1 µ).
	Sediment:	Artificially created by mixing finely ground sphagnum peat moss (720 g), white quartz sand (10,800 g), kaolinite clay (2,880 g), $CaCO_3$ (14.7 g) and deionized water.

Data obtained from p. 17 of the study report.

Table 2: Properties of the water.

Property	Details	
Temperature (°C)	Not reported.	
pH	7.1	
Redox potential (mV)	Not applicable.	
Oxygen concentration (mg/L)	Initial	Final
	5.9	6.1
Dissolved organic carbon (mg/L)	48.1	
Hardness (mg $CaCO_3$/L)	26	
Electrical conductivity (mS/cm)	Not applicable.	
Total dissolved solids (mg/L)	912	
Biomass (cells/mL water)	Not applicable.	

Data obtained from Table 1, p. 30 of the study report.

Table 3: Properties of the sediment[1].

Property		Details
Soil texture		Loamy sand
% Sand		88.7
% Silt		0.7
% Clay		10.6
pH	sediment:water (1:1)	5.8
	0.01M $CaCl_2$	5.1
	Saturated paste	5.7
Organic carbon (%)		3.5
Organic matter (%)		6.1
CEC (meq/100 g)		NA
Redox potential (mV)		Not reported.
Moisture (%)	at 0.33 bar	11.9
	at 15 bar	7.1
Bulk density (g/cm^3)		1.15
Biomass (µg/g sediment)		Not applicable.

1 Sediment was artificially created based on OECD guidance document 218 (p. 16).
Data obtained from Table 1, p. 29 of the study report.

B. EXPERIMENTAL CONDITIONS:

1. Preliminary experiments: No preliminary studies were described.

2. Experimental conditions:

Table 4: Experimental design.

Parameter			Details
Duration of the test			28 days.
Water:			
	Filtered/unfiltered water:		Filtered.
	Type and size of filter used, if any:		5 micron bag filter, granular activated carbon filter and 1 micron cartridge.
Amount of sediment and water per treatment	Water:		349 mL (300 mL water and 49 mL sediment water).
	Sediment:		100 g (dry wt.).
Water/sediment ratios			3.5:1 (300 mL water and 49 mL sediment water:100 g dry wt. sediment).
Application rates (mg a.i./L)	Nominal:		0.09
	Actual:		0.086
Control conditions, if used			Sterile controls were not used.
No. of replications	Control, if used:		Sterile controls were not used.
	Treated:		14 flasks were prepared.
Test apparatus (type/material/volume):			The test apparatus consisted of silanized glass flasks (500 mL, 7.2 cm i.d.) containing treated soil that was flooded and attached to a volatile trapping system. The test apparatus is illustrated in Figure 2, p. 38
Details of traps for CO_2 and organic volatile, if any:			Humidified air was drawn through (10-20 mL/min.) the water in the sample flasks, then through an ethanolamine trap for the collection of CO_2.
If no traps were used, is the system closed?			Volatile traps were used.
Identity and concentration of co-solvent:			Acetonitrile, <0.1%.
Test material application method	Volume of the test solution used/treatment:		≤250 µL.
	Application method:		Test solution was applied to the surface of the sediment using a 250-µL Gilson Microman positive displacement pipette and stirred for 30 minutes using a stir plate. The sediment was then flooded.
Any indication of the test material adsorbing to the walls of the test apparatus?			None.
Microbial biomass/ population of controls (units)		Water:	Sterile controls were not used.
		Sediment:	
Microbial biomass/ population of untreated systems (µg/g)		Water:	Not applicable.
		Sediment:	
Microbial biomass/ population of treated (units)		Water:	Not applicable.
		Sediment:	

Parameter		Details
Experimental conditions:	Temperature (°C):	20.0 ± 0.3°C.
	Continuous darkness (Yes/No):	Yes.
Other details, if any		Sediment was created in the lab.

Data obtained from pp. 17-19, Tables 1-2, pp. 29-32, and Figure 2, p. 38, of the study report.

3. Aerobic conditions: Following treatment, humidified air was continuously bubbled (10-20 mL/min.) into the water layer of the test systems (p. 18). Redox potentials were not measured. Dissolved oxygen in the water layers were 3.8-6.1 mg/L (Table 4, p. 34).

4. Supplementary experiments: No supplementary studies were described.

5. Sampling:

Table 5: Sampling details.

Criteria	Details
Sampling intervals	0, 1, 3, 7, 12, 17, 22 and 28 days.
Sampling method	Single flasks were collected at each interval.
Method of collection of CO_2 and organic volatile compounds	The ethanolamine trap did not need replacement and was collected for analysis at the final sampling interval.
Sampling intervals/times for: Sterility check, if sterile controls are used: Redox potential, dissolved oxygen and pH in water layer and redox potential in sediment:	 Sterile controls were not used. The pH and dissolved oxygen were measured at each sampling interval.
Sample storage before analysis	Water layers and sediment were separated and the sediment was extracted the day of collection. Typically, HPLC was analysis was conducted within 17 days. Extracts and water were stored frozen (<-15°C).
Other details, if any	None reported.

Data obtained from pp. 19-20, 27 and Table 3, p. 33 of the study report.

C. ANALYTICAL METHODS:

Separation of the water and sediment: The water layer was decanted and filtered through a glass fiber filter (Whatman GF/F, 200 µm; p. 20, Figure 4, p. 40). Aliquots were analyzed for total radioactivity by LSC. The sediment was transferred to a Buchner and filtered using a GF/F filter and hydromatrix under vacuum for 30 minutes. Aliquots of the pore water were analyzed by LSC.

Extraction/clean up/concentration methods for water and sediment samples: Sediment samples were transferred to Teflon bottles with acetonitrile:water with 0.1% formic acid (9:1, v:v) and mechanically shaken for 15 minutes (p. 20, Figure 4, p. 40). The extraction was repeated once. Following both extractions, the sediment was centrifuged (10 minutes at 2,900 rpm) and filtered using a GF/F filter. All of the filters were extracted once using the same procedure. The three extracts were combined and aliquots were analyzed for total radioactivity (p. 21).

Extracted sediment was further extracted using a microwave (p. 21). The sediment was mixed with acetonitrile:water with 0.1% formic acid (9:1, v:v) and heated at 80°C for 5 minutes (p. 21). The extraction was repeated once. Following both extractions, the sediment was centrifuged (10 minutes at 2,900 rpm) and filtered using a GF/F filter.

Extracts were transferred to round bottom flasks and concentrated using rotary evaporation at 30°C (p. 21). The extracts were sonicated and reconstituted in acetonitrile and analyzed by LSC and HPLC.

Total ^{14}C measurement: Total ^{14}C residues were determined by summing the concentrations of residues measured in the water layers, sediment extracts, extracted sediment and volatile trapping solutions (Table 5, p. 35).

Determination of nonextractable residues: The extracted sediment was air dried, homogenized and aliquots were analyzed for total radioactivity by LSC following combustion (p. 21, Figure 4, p. 40).

Determination of volatile residues: Triplicate aliquots (1 mL) of the trapping solutions were analyzed for total radioactivity by LSC (p. 20).

Derivatization method, if used: None was reported.

Identification and quantification of parent compound: Water and sediment extract samples were analyzed by HPLC under the following conditions: Phenomenex C18 (4 x 3 mm, 10 µm) guard column, YMC ODS-AQ (4.6 x 250 mm, 5 µm) column, gradient mobile phase combining (A) acetonitrile and (B) 1% formic acid in water [percent A:B at 0 min. 70:30 (v:v), 30 min. 10:90, 35-45 min. 0:100, 50 min. 70:30], flow rate 1.0 mL/minute (pp. 21-22). Parent [^{14}C]spiromesifen was identified by co-chromatography with and comparison to the retention time of unlabeled reference.

LC/ESI/MS was used to confirm HPLC data using a Thermo Electron Quantum Ultra AM mass spectrometer (p. 22). Samples were chromatographed using HPLC with a quaternary system under the following conditions: a Phenomenex Zorbax Rx C8 (150 x 2 mm, 5 µm) column, a linear gradient mobile phase from 95% aqueous formic acid (0.1%):5% methanol to 5% aqueous formic acid (0.1%):95% methanol, and a flow rate of 250 µL/minute.

Detection limits (LOD, LOQ) for the parent compound: The LOD for HPLC was 1000 dpm and the LOQ was 0.3% of the applied (p. 24). The LSC sensitivity limit was twice background (Appendix 3, p. 50). No other values were reported.

Identification and quantification of transformation products: Transformation products were separated and quantified using HPLC and LC/ESI/MS as described for the parent compound (pp. 21-22).

II. RESULTS AND DISCUSSION

A. TEST CONDITIONS The test conditions outlined in the study were reportedly maintained throughout the incubations. The temperature was maintained at 20 ± 0.3°C (p. 19). The pH of the water ranged from 6.7-7.8 and of the sediment from 5.6-6.7; with a pattern of increase (Table 4, p. 34). The dissolved oxygen ranged from 3.8-6.1 mg/L. Redox potentials were not reported.

B. MATERIAL BALANCE: Overall recoveries of [^{14}C]residues averaged 98.9 ± 1.8% (range 95.8-100.6%) of the applied. [^{14}C]Residues moved from the sediment to the water with distribution ratios (sediment:water) of *ca.* 66:1 at time 0, 17:1 at 7 days, and 4:1 at 28 Table 5, p. 35).

Table 6: Biotransformation of [^{14}C]spiromesifen, expressed as percentage of applied radioactivity (n =1), in water-loamy sand sediment under aerobic conditions.

Compound		Sampling times (days)							
		0	**1**	**3**	**7**	**12**	**17**	**22**	**28**
Spiromesifen	Sediment	92.1	93.0	93.1	92.3	87.9	82.5	75.8	74.9
	Water	0.0	0.0	0.7	0.4	0.2	0.4	0.3	0.0
	System	92.1	93.0	93.8	92.7	88.1	82.9	76.1	74.9
BSN2060-enol	Sediment	0.5	0.7	1.1	1.7	3.0	4.3	6.2	5.4
	Water	0.0	0.0	2.5	3.9	5.8	9.7	14.8	16.6
	System	0.5	0.7	3.6	5.6	8.8	14.0	21.0	22.0
Unknown A	Sediment	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Water	0.0	0.0	0.2	0.3	0.4	0.4	0.4	0.4
	System	0.0	0.0	0.2	0.3	0.4	0.4	0.4	0.4
Unidentified	Sediment	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Water[1]	0.3	0.3	0.7	1.2	1.7	2.6	2.8	3.1
	System	0.3	0.3	0.7	1.2	1.7	2.6	2.8	3.1
Total volatiles [2]		--	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Extractable sediment residues		92.5	93.7	94.1	94.0	90.9	86.8	82.0	80.3
Nonextractable sediment residues		1.9	0.3	0.5	0.9	0.4	0.3	0.4	0.5
Total recovery	Water	1.4	2.8	3.9	5.5	7.7	12.7	17.9	19.7
	Sediment	94.4	94.0	94.6	94.9	91.3	87.1	82.4	80.8
	System	95.8	96.9	98.5	100.3	99.0	99.9	100.3	100.6

Data obtained from Tables 5-6, pp. 35-36 of the study report. Totals calculated by the reviewer.

1 Water concentrations include radioactivity in the pore water.

2 Total volatiles was defined by the study author as CO_2, however the trapping solution was ethanolamine.

C. TRANSFORMATION OF PARENT COMPOUND: [^{14}C]Spiromesifen in the sediment decreased from 92.1% of the applied at time 0 to 74.9% at 28 days (Table 6, p. 36). In the water layer, [^{14}C]spiromesifen increased from 0.0% at time 0 to a maximum 0.7% at 3 days, then decreased to 0.0% at 28 days.

HALF-LIFE/DT50/DT90: Based on first order linear regression analysis (Excel 2000), the half-lives of spiromesifen were 80.6 days in the sediment and total system (all intervals); a half-life in the water was not calculated because the highest concentration was 0.7% of the applied (DER Attachment 2). Based on nonlinear analysis (SigmaPlot v 9), half-lives were 82.5 days in the sediment and total system. Observed DT50 and DT90 values were not determined.

The study author did not determine a half-life.

Half-lives/DT50/DT90

Phase	Half-life/DT50 [1] (days)	First order linear regression equation	r^2	DT50 (days)	DT90 (days)
Water					
Linear/natural log	ND	--	--	--	--
Nonlinear/normal	ND	--	--	--	--
Loamy sand					
Linear/natural log	80.6	y = -0.0086x + 4.5526	0.9300	--	--
Nonlinear/normal	82.5	--	0.9995	--	--
Total system					
Linear/natural log	80.6	y = -0.0086x + 4.45556	0.9249	--	--
Nonlinear/normal	82.5	--	0.9994	--	--

ND: Not determined due to the low concentration in water.
1 Determined by the primary reviewer using Excel 2000 (linear) and Sigmaplot v 9 (nonlinear) using Table 6, p. 36.

TRANSFORMATION PRODUCTS: One major transformation product was isolated in the water only (Table 6, p. 36). In the water, BSN2060-enol was a maximum 16.6% of the applied at 28 days. BSN2060-enol was a maximum 6.2% in the sediment at 22 days and 22.0% at 28 days in the total system. No minor transformation products were identified. Unknown A was isolated only in the water at a maximum 0.4% of the applied. Other unidentified radioactivity in the water was a maximum 3.1% of the applied.

NONEXTRACTABLE AND EXTRACTABLE RESIDUES: Extractable [^{14}C]residues increased from 92.5% at time 0 to 94.1% at 3 days and then decreased to 80.3% of the applied at 28 days (Table 5, p. 35). Nonextractable [^{14}C]residues decreased from 1.9% at time 0 to 0.3-0.5% at all other sampling intervals.

VOLATILIZATION: No volatiles were detected in the ethanolamine trap (Table 5, p. 35).

TRANSFORMATION PATHWAY: Spiromesifen degraded to 4-hydroxy-3-(2,4,6-trimethylphenyl)-1-oxaspiro[4.4]non-3-en-2-one (BSN2060-enol).

Table 7: Chemical names and CAS numbers for the transformation products of spiromesifen.

Applicants Code Name	CAS Number	Chemical Name	Chemical Formula	MW (g/mol)	Smiles String
BSN2060-enol	---	4-Hydroxy-3-(2,4,6-trimethylphenyl)-1-oxaspiro[4.4]non-3-en-2-one	---	---	---

Data obtained from Figure 1, p. 37 of the study report.

D. SUPPLEMENTARY EXPERIMENT-RESULTS: No supplementary studies were described.

III. STUDY DEFICIENCIES

1. This experiment was not intended to fulfill a guideline requirement, but to support the request for a waiver of the Sediment Toxicity Study (Draft Guideline 850.1790, Part 2). The matrix used in the study (the water and loamy sand that were created in the laboratory) was prepared based on OECD Guideline 218 (Sediment-Water Chironomid Toxicity Test Using Spiked Sediment; pp. 16, 28).

   Study features that were consistent with the study protocol include:
   a. The water:sediment ratio (3.5:1) approximated the target ratio of 4:1.
   b. The application rate (86 µg/L) was based on a direct overspray of spiromesifen at 900 g a.i./ha to a body of water 100 cm in depth (p. 19).
   c. The sediment was directly treated and mixed for 30 minutes prior to addition of the water layer.
   d. The study was terminated after 28 days, at which time 74.9% of the applied spiromesifen remained undegraded, in accordance with the target duration of 28 days or time of parent DT75.
   e. Mass balance (95.8-100.6%) was between 90-110%.

   Study features that were inconsistent with the study protocol include:
   f. 14 treated and 2 untreated samples were prepared; whereas the protocol called for a minimum of 15 sediment-water systems and 2 water-only systems.
   g. Half-way through the test period of 30 days, paired test systems were not combined and the water and sediment of each combined system were not characterized.

2. The viability of the test system was not reported. Based on the description of the preparation of the system, it is possible that the water and artificial sediment were sterile or nearly sterile at the time the system was prepared. The water was filtered through an ultraviolet sterilizer.

The sediment was prepared using sphagnum peat moss, quartz sand, clay, and calcium carbonate.

3. Only single samples were collected at each sampling interval. It is preferable that replicate samples be collected at each interval.

4. The study author stated that samples were proven to be stable for up to 45 days when stored (p. 27). No supporting data were provided.

IV. REVIEWER'S COMMENTS

1. The study author stated that degradation would be expected to be more rapid in natural water/sediment systems, but did not provide any supporting data (p. 27). Previously submitted aerobic aquatic metabolism studies using natural systems (MRID 45819803, 45819804) are consistent with this claim, with total system degradation half-lives of 12.3 and 17.7 days. However, the difference in degradation rates between the previously submitted studies and this study could be due to many factors, such as system viability, water/sediment source, and treatment method (*i.e.*, whether the water or sediment was spiked).

V. REFERENCES

1. U.S. Environmental Protection Agency. 1982. Pesticide Assessment Guidelines, Subdivision N, Chemistry: Environmental Fate, Section 162-4, Aerobic Aquatic Metabolism Studies. Office of Pesticide and Toxic Substances, Washington, DC. EPA 540/9-82-021.

2. U.S. Environmental Protection Agency. 1989. FIFRA Accelerated Reregistration, Phase 3 Technical Guidance. Office of the Prevention, Pesticides, and Toxic Substances, Washington, DC. EPA 540/09-90-078.

3. U.S. Environmental Protection Agency. 1993. Pesticide Registration Rejection Rate Analysis - Environmental Fate. Office of the Prevention, Pesticides, and Toxic Substances, Washington, DC. EPA 738-R-93-010.

4. Wolfe, N., *et al.* 1990. Abiotic transformations in water, sediments and soil. *In* Pesticides in the Soil Environment, Soil Science Society of America, pp. 103-110.

Attachment 1: Structures of Parent Compound and Transformation Products

Spiromesifen [C-798D, BSN 2060]

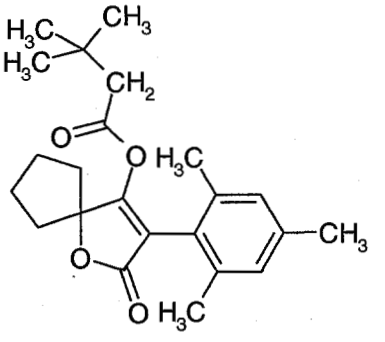
IUPAC Name: 3-Mesityl-2-oxo-1-oxaspiro[4.4]non-3-en-4-yl 3,3-dimethylbutyrate.

CAS Name: 2-Oxo-3-(2,4,6-trimethylphenyl)-1-oxaspiro[4.4]non-3-en-4-yl 3,3-dimethylbutanoate.

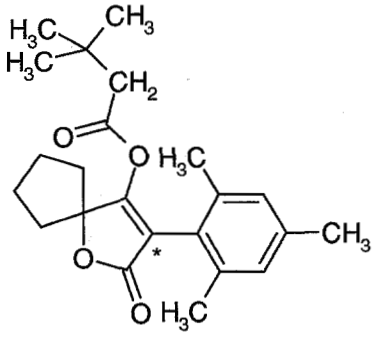
CAS Number: 283594-90-1.

SMILES String: C32(CCCC3)C(OC(=O)CC(C)(C)C)=C(C1C(C)=CC(C)=CC=1C)C(=O)O 2 (EPI Suite, v3.12 SMILES string from ISIS .MOL).
O=C(CC(C)(C)C)OC1=C(c2c(cc(cc2C)C)C)C(=O)OC11CCCC1 (SMILES String provided by study author).

Unlabeled



[Dihydrofuranone-3-^{14}C]Spiromesifen



* = Location of the radiolabel.

Identified Compounds

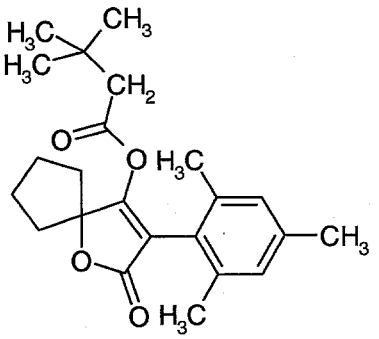
Spiromesifen [C-798D, BSN 2060]

IUPAC Name: 3-Mesityl-2-oxo-1-oxaspiro[4.4]non-3-en-4-yl 3,3-dimethylbutyrate.

CAS Name: 2-Oxo-3-(2,4,6-trimethylphenyl)-1-oxaspiro[4.4]non-3-en-4-yl 3,3-dimethylbutanoate.

CAS Number: 283594-90-1.

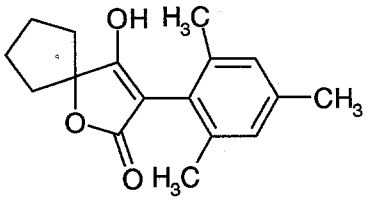
SMILES String: C32(CCCC3)C(OC(=O)CC(C)(C)C)=C(C1C(C)=CC(C)=CC=1C)C(=O)O 2 (EPI Suite, v3.12 SMILES string from ISIS .MOL).
O=C(CC(C)(C)C)OC1=C(c2c(cc(cc2C)C)C)C(=O)OC11CCCC1 (SMILES String provided by study author).



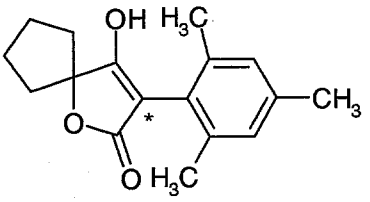
BSN2060-enol [C-799A]

IUPAC Name: Not reported.
CAS Name: 4-Hydroxy-3-(2,4,6-trimethylphenyl)-1-oxaspiro[4.4]non-3-en-2-one.
CAS Number: 148476-30-6.
SMILES String: C32(CCCC3)C(O)=C(C1C(C)=CC(C)=CC=1C)C(=O)O2 (EPI Suite, v3.12 SMILES string from ISIS .MOL).
OC1=C(c2c(cc(cc2C)C)C)C(=O)OC11CCCC1 (SMILES String provided by study author).

Unlabeled



[Dihydrofuranone-3-^{14}C]BSN2060-enol



* = Location of the radiolabel.

Attachment 2: Excel and SigmaPlot Spreadsheets

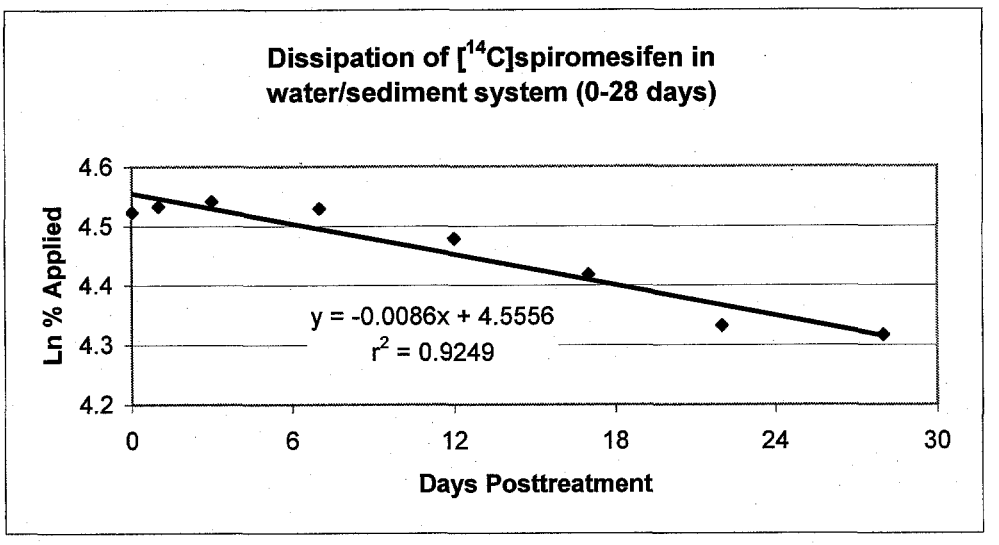
Chemical: Spiromesifen
MRID:47133102
PC: 024875
Guideline: 162-4

Total system
Half-life (days): 80.6

Nonlinear (days) **82.5**
r^2 = 0.9994

Day	Spiromesifen (% of applied)	Ln (% applied)
0	92.1	4.5229
1	93.0	4.5326
3	93.8	4.5412
7	92.7	4.5294
12	88.1	4.4785
17	82.9	4.4176
22	76.1	4.3320
28	74.9	4.3162

Data obtained from Table 6, p. 36 of the study report.



SUMMARY OUTPUT

Regression Statistics	
Multiple R	0.961726565
R Square	0.924917985
Adjusted R Squa	0.912404316
Standard Error	0.027357979
Observations	8

ANOVA

	df	*SS*	*MS*	*F*	*ignificance F*
Regression	1	0.055320563	0.055320563	73.91261	0.000136
Residual	6	0.004490754	0.000748459		
Total	7	0.059811317			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*ower 95.0%*	*pper 95.0%*
Intercept	4.555570715	0.014841909	306.9396788	8.07E-14	4.519254	4.591888	4.519254	4.591888
X Variable 1	-0.00860276	0.001000641	-8.59724452	0.000136	-0.01105	-0.00615	-0.01105	-0.00615

Chemical: Spiromesifen
MRID:47133102
PC: 024875
Guideline: 162-4

Sediment

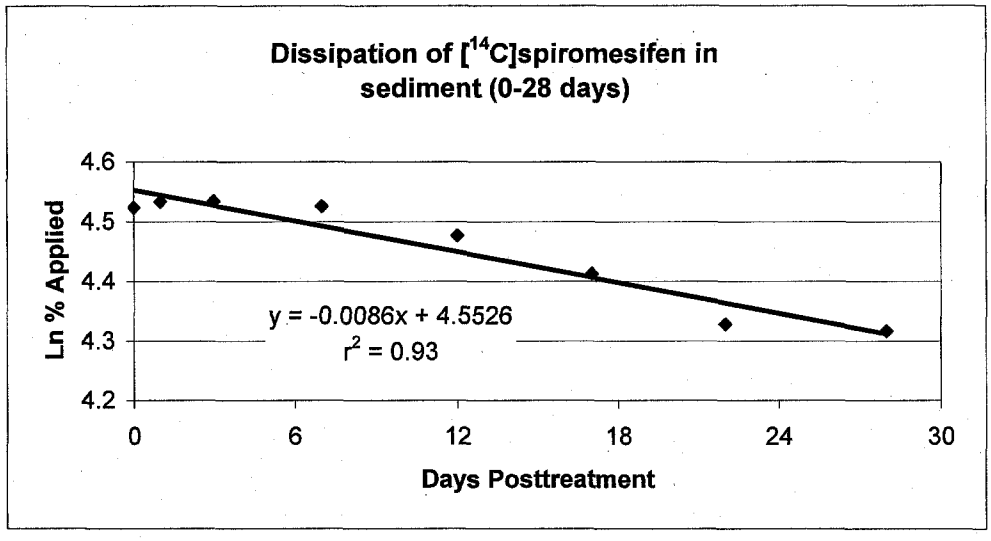
Half-life (days): 80.6

Nonlinear (days) **82.5**

r^2 = 0.9995

Day	Spiromesifen (% of applied)	Ln (% applied)
0	92.1	4.5229
1	93.0	4.5326
3	93.1	4.5337
7	92.3	4.5250
12	87.9	4.4762
17	82.5	4.4128
22	75.8	4.3281
28	74.9	4.3162

Data obtained from Table 6, p. 36 of the study report.



SUMMARY OUTPUT

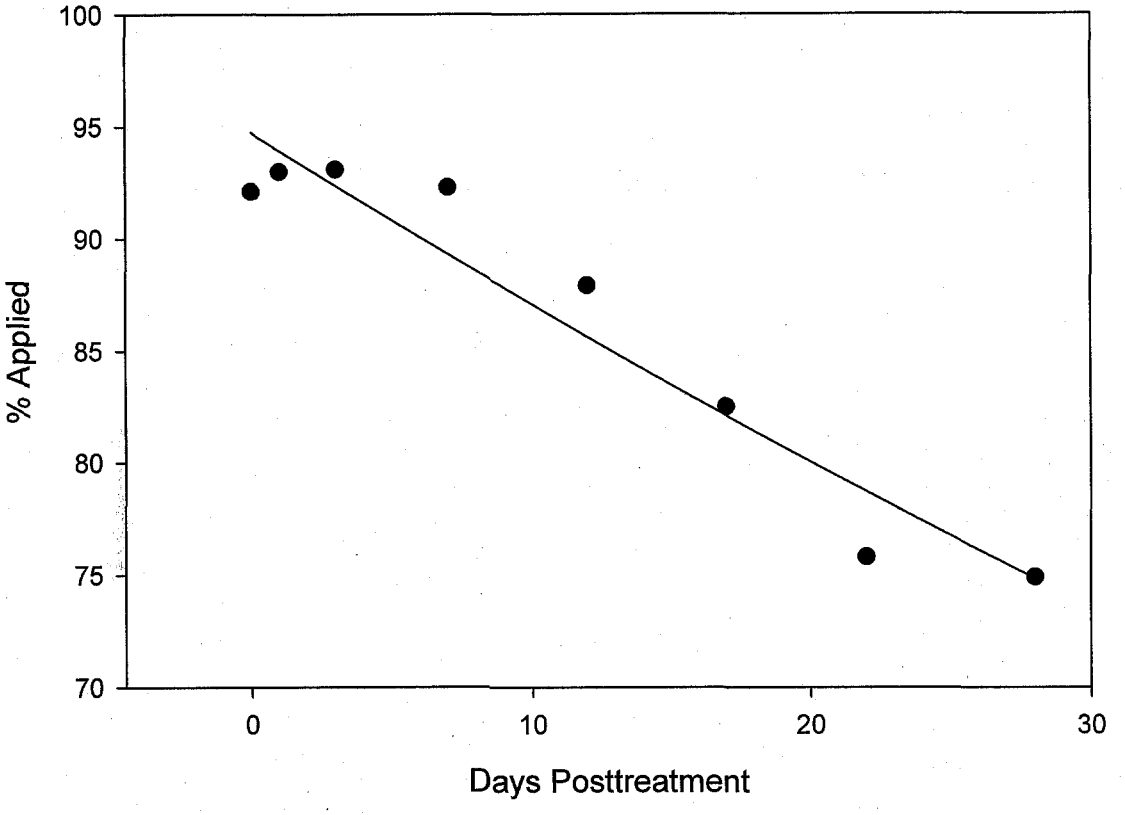
Regression Statistics	
Multiple R	0.964386414
R Square	0.930041155
Adjusted R Squa	0.918381348
Standard Error	0.026301703
Observations	8

ANOVA

	df	*SS*	*MS*	*F*	*ignificance F*
Regression	1	0.055179597	0.055179597	79.76471	0.00011
Residual	6	0.004150677	0.00069178		
Total	7	0.059330275			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*ower 95.0%*	*pper 95.0%*
Intercept	4.55258802	0.014268871	319.0573407	6.4E-14	4.517673	4.587503	4.517673	4.587503
X Variable 1	-0.00859179	0.000962007	-8.9311091	0.00011	-0.01095	-0.00624	-0.01095	-0.00624

Dissipation of [^{14}C]spiromesifen
in artificial loamy sand sediment



Nonlinear Regression

Data Source: Data 1 in Notebook1
Equation: Single, 2 Parameter

R	Rsqr	Adj Rsqr	Standard Error of Estimate
0.9997	0.9995	0.9993	2.2766

	Coefficient	Std. Error	t	P	VIF
a	94.7381	1.2755	74.2740	<0.0001	2.1046
b	0.0084	0.0010	8.4107	0.0002	2.1046

Analysis of Variance:

	DF	SS	MS
Regression	2	60175.5230	30087.7615
Residual	6	31.0970	5.1828
Total	8	60206.6200	7525.8275

Statistical Tests:

PRESS 53.0751

Durbin-Watson Statistic 1.1137 Failed

Normality Test Passed (P = 0.9922)

K-S Statistic = 0.1446 Significance Level = 0.9922

Constant Variance Test Passed (P = 0.4228)

Power of performed test with alpha = 0.0500: 1.0000

Regression Diagnostics:

Row	Predicted	Residual	Std. Res.	Stud. Res.	Stud. Del. Res.
1	94.7381	-2.6381	-1.1588	-1.3990	-1.5558
2	93.9431	-0.9431	-0.4143	-0.4872	-0.4539
3	92.3732	0.7268	0.3192	0.3607	0.3329
4	89.3117	2.9883	1.3126	1.4188	1.5889
5	85.6271	2.2729	0.9984	1.0698	1.0857
6	82.0945	0.4055	0.1781	0.1968	0.1802
7	78.7076	-2.9076	-1.2772	-1.5087	-1.7482
8	74.8273	0.0727	0.0319	0.0432	0.0395

Influence Diagnostics:

Row	Cook's Dist	Leverage	DFFITS
1	0.4477	0.3139	-1.0524
2	0.0455	0.2771	-0.2810
3	0.0180	0.2168	0.1751
4	0.1695	0.1441	0.6520
5	0.0848	0.1291	0.4180
6	0.0043	0.1809	0.0847

7	0.4501	0.2834	-1.0994
8	0.0008	0.4547	0.0361

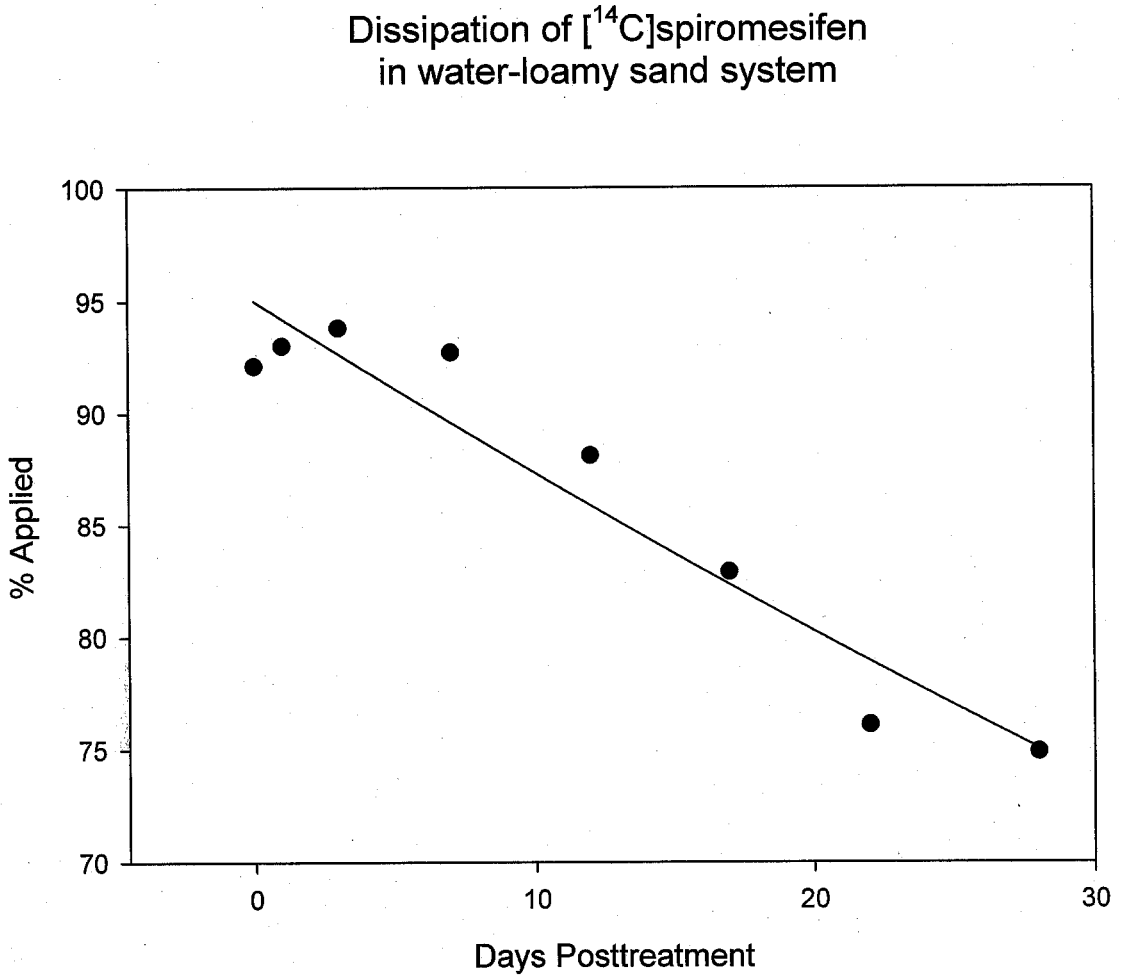
95% Confidence:

Row	Predicted	Regr. 5%	Regr. 95%	Pop. 5%	Pop. 95%
1	94.7381	91.6170	97.8591	88.3527	101.1234
2	93.9431	91.0108	96.8755	87.6479	100.2384
3	92.3732	89.7796	94.9669	86.2284	98.5180
4	89.3117	87.1969	91.4265	83.3532	95.2702
5	85.6271	83.6254	87.6288	79.7078	91.5464
6	82.0945	79.7254	84.4635	76.0411	88.1479
7	78.7076	75.7422	81.6730	72.3969	85.0184
8	74.8273	71.0708	78.5838	68.1085	81.5461

Fit Equation Description:

[Variables]
x = col(1)
y = col(2)
reciprocal_y = 1/abs(y)
reciprocal_ysquare = 1/y^2
'Automatic Initial Parameter Estimate Functions
F(q)=if(size(x)>1, if(total(abs(y))>0, ape(x,log(abs(y)),1,0,1), -306), 0)
asign(q)=if(mean(q)>=0,1,-1)
[Parameters]
a = if(F(0)[1]< 307, if(F(0)[1]>-307, asign(y)*10^F(0)[1], asign(y)*10^(-307)), asign(y)*10^307) "Auto {{previous: 94.7381}}
b = if(x50(x,y)-min(x)=0, 1, -ln(.5)/(x50(x,y)-min(x))) "Auto {{previous: 0.00842617}}
[Equation]
f = a*exp(-b*x)
fit f to y
"fit f to y with weight reciprocal_y
"fit f to y with weight reciprocal_ysquare
[Constraints]
b>0
[Options]
tolerance = 1e-10
stepsize = 1
iterations=200

Number of Iterations Performed = 7


Dissipation of [14C]spiromesifen
in water-loamy sand system
100
95
90
85
80
75
70
% Applied
0
10
20
30
Days Posttreatment

Nonlinear Regression

Data Source: Data 1 in Notebook2
Equation: Single, 2 Parameter

R	Rsqr	Adj Rsqr	Standard Error of Estimate
0.9997	0.9994	0.9992	2.3955

	Coefficient	Std. Error	t	P	VIF
a	95.0060	1.3421	70.7877	<0.0001	2.1048
b	0.0084	0.0011	8.0105	0.0002	2.1048

Analysis of Variance:

	DF	SS	MS
Regression	2	60523.9490	30261.9745
Residual	6	34.4310	5.7385
Total	8	60558.3800	7569.7975

Statistical Tests:

PRESS 59.1305

Durbin-Watson Statistic 1.0105 Failed

Normality Test Passed (P = 0.9881)

K-S Statistic = 0.1499 Significance Level = 0.9881

Constant Variance Test Passed (P = 0.2327)

Power of performed test with alpha = 0.0500: 1.0000

Regression Diagnostics:

Row	Predicted	Residual	Std. Res.	Stud. Res.	Stud. Del. Res.
1	95.0060	-2.9060	-1.2131	-1.4645	-1.6679
2	94.2094	-1.2094	-0.5049	-0.5938	-0.5587
3	92.6363	1.1637	0.4858	0.5489	0.5142
4	89.5683	3.1317	1.3073	1.4131	1.5793
5	85.8759	2.2241	0.9285	0.9949	0.9939
6	82.3356	0.5644	0.2356	0.2603	0.2390
7	78.9414	-2.8414	-1.1861	-1.4011	-1.5594
8	75.0524	-0.1524	-0.0636	-0.0862	-0.0787

Influence Diagnostics:

Row	Cook's Dist	Leverage	DFFITS
1	0.4907	0.3139	-1.1282
2	0.0676	0.2771	-0.3459
3	0.0417	0.2168	0.2705
4	0.1681	0.1441	0.6481
5	0.0734	0.1291	0.3827
6	0.0075	0.1809	0.1123

7	0.3882	0.2834	-0.9806
8	0.0031	0.4548	-0.0719

95% Confidence:

Row	Predicted	Regr. 5%	Regr. 95%	Pop. 5%	Pop. 95%
1	95.0060	91.7219	98.2901	88.2871	101.7249
2	94.2094	91.1239	97.2949	87.5853	100.8336
3	92.6363	89.9071	95.3654	86.1705	99.1021
4	89.5683	87.3430	91.7936	83.2985	95.8381
5	85.8759	83.7696	87.9821	79.6473	92.1044
6	82.3356	79.8429	84.8284	75.9660	88.7053
7	78.9414	75.8210	82.0617	72.3009	85.5818
8	75.0524	71.0995	79.0053	67.9825	82.1223

Fit Equation Description:

[Variables]
x = col(1)
y = col(2)
reciprocal_y = 1/abs(y)
reciprocal_ysquare = 1/y^2
'Automatic Initial Parameter Estimate Functions
F(q)=if(size(x)>1, if(total(abs(y))>0, ape(x,log(abs(y)),1,0,1), -306), 0)
asign(q)=if(mean(q)>=0,1,-1)
[Parameters]
a = if(F(0)[1]< 307, if(F(0)[1]>-307, asign(y)*10^F(0)[1], asign(y)*10^(-307)), asign(y)*10^307) "Auto {{previous: 95.006}}
b = if(x50(x,y)-min(x)=0, 1, -ln(.5)/(x50(x,y)-min(x))) "Auto {{previous: 0.00841977}}
[Equation]
f = a*exp(-b*x)
fit f to y
"fit f to y with weight reciprocal_y
"fit f to y with weight reciprocal_ysquare
[Constraints]
b>0
[Options]
tolerance = 1e-10
stepsize = 1
iterations=200

Number of Iterations Performed = 7

Chemical: Spiromesifen
MRID:47133102
PC: 024875
Guideline: 162-4

Material Balance

Days Posttreatment	Total % Applied
0	95.8
1	96.9
3	98.5
7	100.3
12	99.0
17	99.9
22	100.3
28	100.6
average	98.9
sd	1.8

Data obtained from Table 5, p. 35 in the study report.

[^{14}C]Residue water phase:soil ratios.

Time	Water % AR	Sediment % AR	Ratio W:S	Ratio S:W
0	1.4	92.5	0.0	66.1
1	2.8	93.7	0.0	33.5
3	3.9	94.1	0.0	24.1
7	5.5	94.0	0.1	17.1
12	7.7	90.9	0.1	11.8
17	12.7	86.8	0.1	6.8
22	17.9	82.0	0.2	4.6
28	19.7	80.3	0.2	4.1

HPLC Analysis

Days Posttreatment	Spiromesifen % applied Water	Sediment	Total	Enol % applied Water	Sediment	Total
0	0.0	92.1	92.1	0.0	0.5	0.5
1	0.0	93.0	93.0	0.0	0.7	0.7
3	0.7	93.1	93.8	2.5	1.1	3.6
7	0.4	92.3	92.7	3.9	1.7	5.6
12	0.2	87.9	88.1	5.8	3.0	8.8
17	0.4	82.5	82.9	9.7	4.3	14.0
22	0.3	75.8	76.1	14.8	6.2	21.0
28	0.0	74.9	74.9	16.6	5.4	22.0

Data obtained from Table 6, p. 36 in the study report.

Attachment 3: Transformation Pathway Presented by Registrant
Illustration of Test System

Figure 2. Aerobic water-sediment test apparatus for spiromesifen.

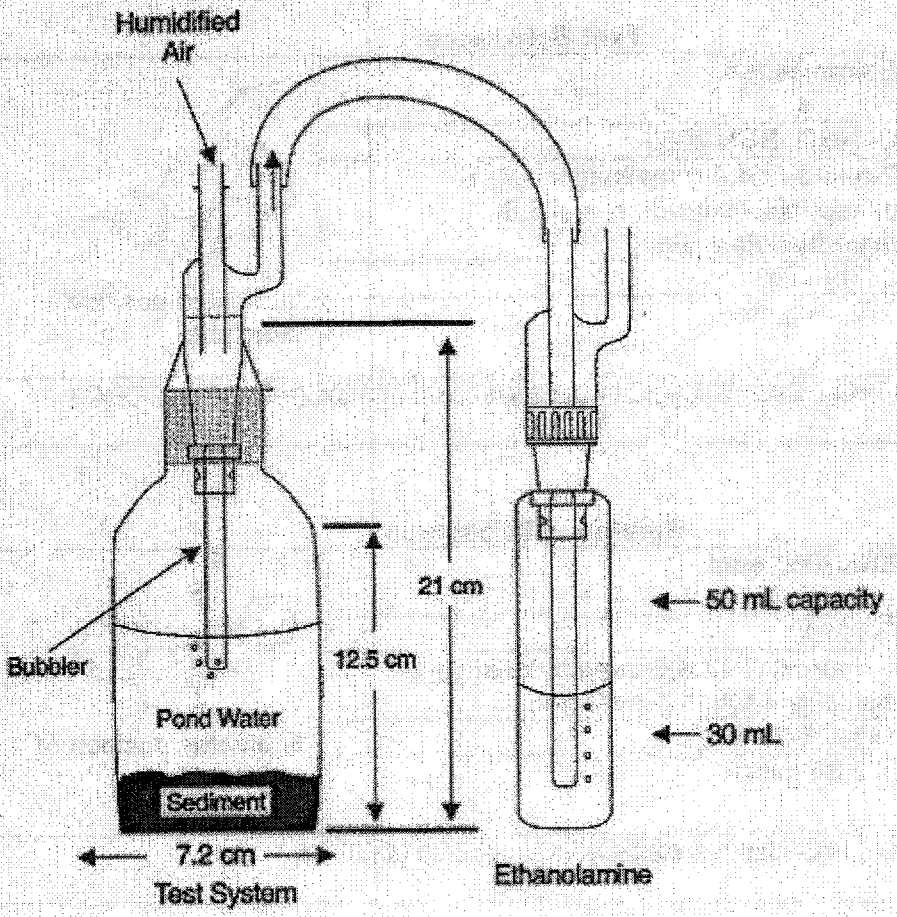


Figure 11. Pathway for spiromesifen in an artificial water-sediment system under aerobic conditions.